

Immune enhancing effects of dehydroepiandrosterone and dehydroepiandrosterone sulphate and the role of steroid sulphatase

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SUMMARY

Steroid hormones, such as glucocorticoids (GC), influence immune and inflammatory responses through their suppressive actions. Recent evidence suggests that another steroid hormone, dehydroepiandrosterone (DHEA), provides an immunostimulatory influence opposing the effect of GC. DHEA circulates in its inactive sulphated form, DHEAS, requiring conversion to DHEA by a steroid sulphatase (SS) enzyme for biological activity. Therefore, inhibition of SS activity may affect immune responses, allowing endogenous GC effects to predominate. We have shown that administration of DHEA and DHEAS in contact sensitization (CS) augments ear swelling by 39 and 46% respectively ($P < 0.001$). DHEAS at doses of 0.5, 5 and 50 mg kg reverses the inhibitory effect of corticosterone (5 mg kg) ($P < 0.01$). In CS, CT2251 (SS inhibitor) at 10 and 0.1 mg kg inhibited ear swelling by 61 and 38% ($P < 0.05$) respectively. In addition, it inhibited DHEAS-augmented responses by 49 and 35% respectively ($P < 0.05$), with no effect on DHEA-augmented responses. DHEAS reversed CT2251 inhibition of the CS response with complete reversal at 50 mg kg ($P < 0.05$). DHEAS and CT2251 appear to affect cellular infiltration into the ear, since DHEAS increased the number of lymphocytes by 63.8% and macrophages by 107% ($P < 0.001$), whereas CT2251 at 0.1 mg kg decreased the number of lymphocytes by 65% ($P < 0.001$) and macrophages by 80% ($P < 0.001$). DHEAS, CT2251 and dexamethasone had no effect on oedema in the ear. From our data we have shown that steroid hormones, such as DHEA, have the potential to act as immunostimulatory factors *in vivo*. Inhibiting the conversion of DHEAS to DHEA by SS enzyme leads to an anti-inflammatory effect.

INTRODUCTION

Dehydroepiandrosterone (DHEA) is an adrenal androgen in man and other species, although in rodents it is likely to be of extra-adrenal origin, since rodent adrenals lack the 17-hydroxylase enzyme.¹ In all species, DHEA is formed from its common precursors, cholesterol and pregnenolone,² and displays circadian rhythmicity.^{3,4} DHEA has intrinsic biological activity but is rapidly sulphated in the adrenal cortex and other tissues to DHEA sulphate (DHEAS).⁵ In the periphery DHEAS lacks biological activity, therefore it must be hydrolysed to DHEA by a steroid sulphatase (SS) enzyme to elicit a biological effect. Amongst its postulated biological roles, DHEA can inhibit glucose-6-phosphate dehydrogenase,⁶ act as a potential precursor for other steroid hormones^{1,2,7} and reverse the insulin resistance associated with glucocorticoid

treatment in genetically obese animals.^{8–10} What remains unclear, however, is whether any of these proposed functions of DHEA are of physiological importance given that it circulates as DHEAS in millimolar concentrations.^{4,11–13} Interest in the effects of DHEA on immune and inflammatory reactions was stimulated by the work of Daynes *et al.*,¹⁴ who showed that splenocytes removed from mice treated with exogenous DHEA or DHEAS showed an increased ability to secrete interleukin-2 (IL-2) but not IL-4 in response to mitogen or antigen (T helper (Th1)-type cytokine profile). These observations contrasted to the effects seen with glucocorticoid (GC) administration which elicited an enhanced IL-4 but reduced IL-2 secretory patterns from splenocytes (T helper (Th2)-type cytokine profile).¹⁵ If DHEA and GC were administered concomitantly, the DHEA effect predominated. The authors concluded that, unlike GC which are immunosuppressive, DHEA is an immunostimulant. The fact that exogenous DHEAS, which lacks biological activity, can elicit biological effects indicates that SS may be an important enzyme in these responses. These, and similar observations, have led to the proposal that ongoing immune responses, in terms of the T-cell cytokine profile (Th1 or Th2-like), can be influenced by the opposing effects of DHEA (or related steroids) and

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Abbreviations: CS, contact sensitization; DEX, dexamethasone; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulphate; GC, glucocorticoids; SS, steroid sulphatase.

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GC.¹⁴⁻²⁰ Furthermore, it could be the activity of the SS enzyme which determines this effect by regulating the availability of DHEA (from inactive DHEAS) within secondary lymphoid tissues. Indeed, the amount of SS correlates anatomically with those lymphoid areas which normally give rise to a high IL-2 T-cell cytokine profile (spleen, peripheral lymph nodes (LN)) and not those that give a low IL-2 high IL-4 profile (Peyer's patches).¹⁶

The aims of the present study were twofold: first, to ascertain whether the immunostimulatory effects seen with exogenous DHEAS administration on cellular responses translates into *in vivo* physiological effects, and secondly, to investigate our postulate that inhibition of SS activity may be anti-inflammatory by allowing endogenous GC effects to predominate. We have addressed these in a mouse contact sensitization model (a form of delayed-type hypersensitivity (DTH)) to show that exogenous DHEA and DHEAS do indeed enhance the inflammatory response to antigen. DTH is generally regarded as being a Th1-type immune reaction. We have used a specific inhibitor of SS (estrone-3-sulphamate, CT2251) in this model to determine the consequences of limiting the conversion of inactive DHEAS to active DHEA. Estrone-3-sulphamate (CT2251) has been shown to be a potent (K_i 0.67 μ M), irreversible, active site-directed inhibitor of steroid sulphatase, a microsomal enzyme.^{21,22} The data indicate that SS is a physiologically important enzyme in these responses and that DHEA (or related steroids) are the likely effector hormones.

MATERIALS AND METHODS

In vivo characterization of steroid sulphatase inhibitor activity

Because of the irreversible binding nature of oestrone-3-sulphamate (CT2251), its likely duration of action was estimated by treating male BALB c mice (16–20 g) (Harlan UK Ltd, Bicester, UK) with the drug and measuring liver SS activity at various time points thereafter. CT2251 was given at doses from 0.03 mg kg to 1 mg kg subcutaneously (s.c.) on day 0, and the animals were killed on days 1 to 4 post-administration. In addition, animals treated with doses of CT2251 from 0.003 to 10 mg kg during a CS experiment were killed and liver SS activity was measured prechallenge on day 4 (see below for details of model).

Livers were suspended in four times wet weight of phosphate-buffered saline (PBS), pH 7.2, 250 mM sucrose and hand homogenized on ice. The resulting extracts were centrifuged (10 min, 10 000 g, 4 °C) and the supernatants (containing the microsomal fraction) assayed for DHEAS sulphatase activity using an adapted form of the method of Purohit *et al.*²² Supernatant (containing approximately 0.5 mg protein) was incubated with [³H]DHEAS (3×10^6 , NEN-Dupont, Boston, MA) adjusted to 1 μ M with unlabelled substrate (Sigma Chemical Co., Poole, UK) in PBS, pH 7.2, 250 mM sucrose (total volume 1 ml) for 30 min at 30 °C. The reaction was stopped and [³H]DHEA formed was isolated by extraction with toluene (2 ml) using [¹⁴C]DHEA to monitor recovery. Protein concentrations were determined by the method of Bradford.²³

Effect of steroids and CT2251 on contact sensitization (CS)

The CS model allows the effect of agents that may either augment or inhibit the ear swelling response to be studied.

One day prior to the start of the experiment, male BALB c mice (16–20 g) (Harlan UK Ltd) were shaved on their right flank. On day 0 the mice were painted on the shaven flank with 50 μ l of 2.5% oxazolone (Sigma Chemicals Ltd) in 4:1 acetone:olive oil or vehicle only (4:1 acetone:olive oil). On day 5 the animals were challenged on their right dorsal ear surface with 25 μ l of 0.25% oxazolone unless otherwise stated. Prior to ear challenge, measurements of right and left ear thickness were taken using engineer micrometers. Further ear measurements were taken 24 hr postchallenge, which we have shown to be the time of maximal ear swelling. Controls included animals which were challenged only. Results are expressed as change in ear thickness from the prior measurement. The changes in ear thickness from individual mice were further analysed to give per cent inhibition from vehicle or positive control group using the following formula:

$$\% \text{ inhibition} = \frac{\text{change in ear thickness} - \text{challenge only} \\ (\text{mean of group}) (\text{Individual mice})}{\text{vehicle control (mean of group)} \\ - \text{challenge only (mean)}}$$

In a pilot study we determined that the challenge dose of oxazolone was critical in determining the ear swelling: 2.5% giving the maximal response and 0.25% (the dose used in these experiments) giving approximately 50% maximal response. Steroids (DHEA, DHEAS), dexamethasone (DEX), corticosterone (Sigma Chemical Ltd) or CT2251 were administered s.c. at doses stated later in text, in olive oil or 20% dimethylsulphoxide (DMSO) 80% olive oil generally on day 0 and 4. The following studies were carried out: effect of DHEA, DHEAS and DEX; reversal of corticosterone inhibition; dose response to CT2251; effect of steroid sulphatase inhibition; and reversal of CT2251 inhibition with DHEAS.

Effect of DHEA, DHEAS and CT2251 on the cellular infiltrate in the ear

Contact sensitization was performed in male BALB c mice ($n=10$ per group) as described above. Animals were treated with either DHEAS (5 mg kg), DEX (5 mg kg), CT2251 (0.1 mg kg) or vehicle given s.c. in olive oil on day 0 and 4. Twenty-four hours postchallenge, the ear swelling response was measured, the animals killed, the ears removed, snap-frozen and stored at -70 °C for immunohistochemical analysis. Controls from normal and challenge only animals were included. Cryostat sections (6 μ m) were fixed in acetone for 10 min at room temperature, and, stained for T cells and macrophages using anti-CD3 monoclonal antibody (mAb) (Serotec Ltd, Oxford, UK) and anti-Mac 1 mAb (Serotec Ltd) respectively. Positive staining was visualized using the alkaline-phosphatase anti-alkaline phosphatase (APAAP) technique (Dako Ltd, High Wycombe, UK). Numbers of positive cells were counted blind in 10 random fields per tissue section and expressed as numbers per mm of skin.

Effect of DHEAS, CT2251 and DEX on oedema in the ear

Contact sensitization was performed in male BALB c mice as described above, and the animals were treated with either DHEAS (5 mg kg) and or CT2251 (10 mg kg), DEX (5 mg kg) or vehicle given s.c. in olive oil on day 0 and 4. Ear measurements were taken prior to, and 24 hr postchallenge. At 24 h, ears were excised, and weight measurements made

prior to and after drying in an oven overnight (120 °C), to estimate tissue water content. Results were expressed as change from prior weight. Controls were included from normal and challenge only animals.

Statistical analysis

Data are expressed as per cent inhibition of treated groups from either positive or vehicle control as discussed in the CS section. Statistical analysis was performed by analysis of variance (ANOVA) on the ear thickness measurements (raw data). Differences were considered significant if $P < 0.05$.

Preparation of CT2251

Oestrone-3-sulphamate (CT2251) was prepared by the method of Howarth *et al.*²¹ by treating the sodium salt of oestrone with sulphamoyl chloride²⁴ in anhydrous dimethylformamide. The product was recrystallized from methanol and exhibited satisfactory spectroscopic and microanalytical data.

RESULTS

Characterization of steroid sulphatase inhibition

The irreversible binding of the inhibitor, CT2251, to SS allowed direct measurement of its systemic effect on SS activity in mice treated with the drug. Liver SS was measured due to its relative abundance compared to other tissues.²⁵ Mean specific activity of SS in untreated mice was 0.26 pmol DHEAS converted per minute per mg protein ($n = 36$), with a normal range of activity from 0.2 pmol min⁻¹ mg⁻¹ to 0.32 pmol min⁻¹ mg⁻¹. Treatment of mice with varying doses of CT2251 on day 0 and measurement of liver SS activity on subsequent days revealed that at 1 and 0.1 mg kg SS activity was below the normal range for 3 and 1 days respectively. Examination of liver SS activities in animals treated with 1, 0.1 and 0.01 mg kg CT2251 during the CS model resulted in enzyme activities below the normal range of 0, 0.021 and 0.107 pmol min⁻¹ mg⁻¹ respectively ($P < 0.05$).

Contact sensitization

Effect of DHEA, DHEAS and DEX in contact sensitization (Fig. 1). DHEA and DHEAS at 5 mg kg augmented the CS response with an increase in ear thickness of 39.6% and 45.9% respectively ($P \leq 0.001$ from positive and vehicle control) when given on day 0, 4 and 5. In contrast, DEX at 5 mg kg effectively inhibited the response by 78.8% ($P \leq 0.001$). In subsequent experiments similar results were seen when the steroid hormones were given on day 0 and 4 (data not shown).

Reversal of corticosterone inhibition with increasing doses of DHEAS (Fig. 2). Corticosterone alone inhibited the response by 42.7%, while, DHEAS alone given on day 0 and 4 in 20% DMSO:80% olive oil at 50 and 5 mg kg augmented the CS response by 19.0 and 9.7% respectively. Increasing doses of DHEAS from 0.0005 to 50 mg kg reversed the inhibitory effect of corticosterone in a dose-dependent manner ($P < 0.01$).

Dose response curve to CT2251 (Table 1). CT2251 given s.c. on day 0 and 4 in olive oil at 0.3 and 0.1 mg kg inhibited the CS response by 42.9% and 40.9% ($P < 0.05$ from vehicle control) respectively. Doses below 0.1 mg kg had no suppressive effect on the CS response.

Effect of irreversible sulphatase inhibitor (CT2251) alone,

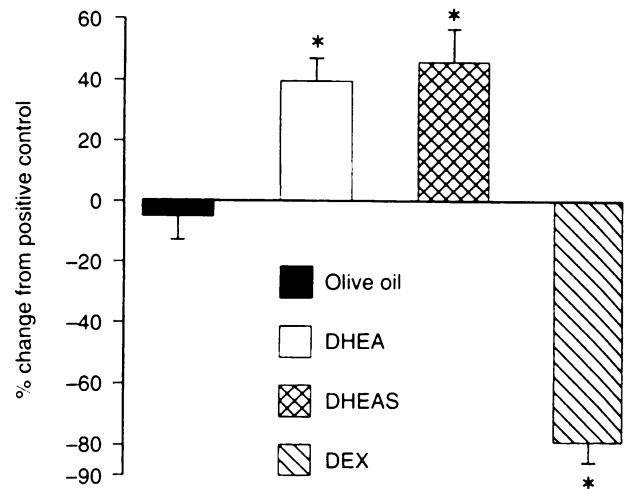


Figure 1. Effect of DHEA, DHEAS, DEX and vehicle in CS. Animals were sensitized with 2.5% oxazolone on day 0, and challenged with 0.25% oxazolone on day 5. Steroids were given on days 0, 4 and 5 s.c. at 5 mg kg. Ear measurements were taken at 24 h postchallenge, and expressed as percentage change from positive control. Change in ear thickness from prior measurement in positive control = 0.136 mm and challenge only control = 0.006 mm. Mean \pm SEM, $n = 7-8$ per group. * $P < 0.001$ from positive control.

and, on DHEA and DHEAS-augmented responses (Fig. 3). CT2251 given on day 0 and 4 in olive oil at 10 and 0.1 mg kg inhibited the CS response by 61.6 and 38.6% respectively ($P < 0.05$ from vehicle control). In contrast, DHEA and DHEAS given on day 0 and 4 in olive oil at 5 mg kg augmented the CS response by 43.1 and 45% respectively ($P < 0.05$). When given in combination with DHEA (5 mg kg), CT2251 at 10 and 0.1 mg kg had no effect on the DHEA-augmented response. However, when given in combination with DHEAS (5 mg kg), CT2251 reversed the DHEAS-augmented CS response ($P < 0.05$).

Reversal of CT2251 inhibition by DHEAS (Fig. 4). In order to investigate whether the effect of CT2251 could be overcome by increasing concentrations of exogenous DHEAS, CT2251 at 0.1 mg kg and DHEAS at 5, 15 and 50 mg kg were given in olive oil on day 0 and 4. The inhibitory effect of 0.1 mg kg CT2251 (51.3%, $P < 0.01$) could not be reversed by 5 mg kg DHEAS, was partially reversed by 15 mg kg DHEAS and completely reversed by 50 mg kg DHEAS ($P < 0.05$).

Effect of CT2251 on cellular infiltrate in the ear

DHEAS at 5 mg kg given in olive oil on day 0 and 4 increased the numbers of both immune (CD3-positive T cells) and inflammatory cells (Mac-1-positive macrophages) compared to vehicle control ($P < 0.001$) (Table 2a). DEX at 5 mg kg effectively inhibited the influx of both lymphocytes (66.8%, $P < 0.001$), and, macrophages (90.1%, $P < 0.001$). In a separate experiment, CT2251 at 0.1 mg kg decreased the number of T cells by 65.3% and macrophages by 80.3%, when compared to vehicle control ($P < 0.001$) (Table 2b).

Effect of CT2251 on oedema in the ear (Table 3)

The percentage water content in a normal mouse ear was 55.4%. In animals which had been both sensitized and chal-

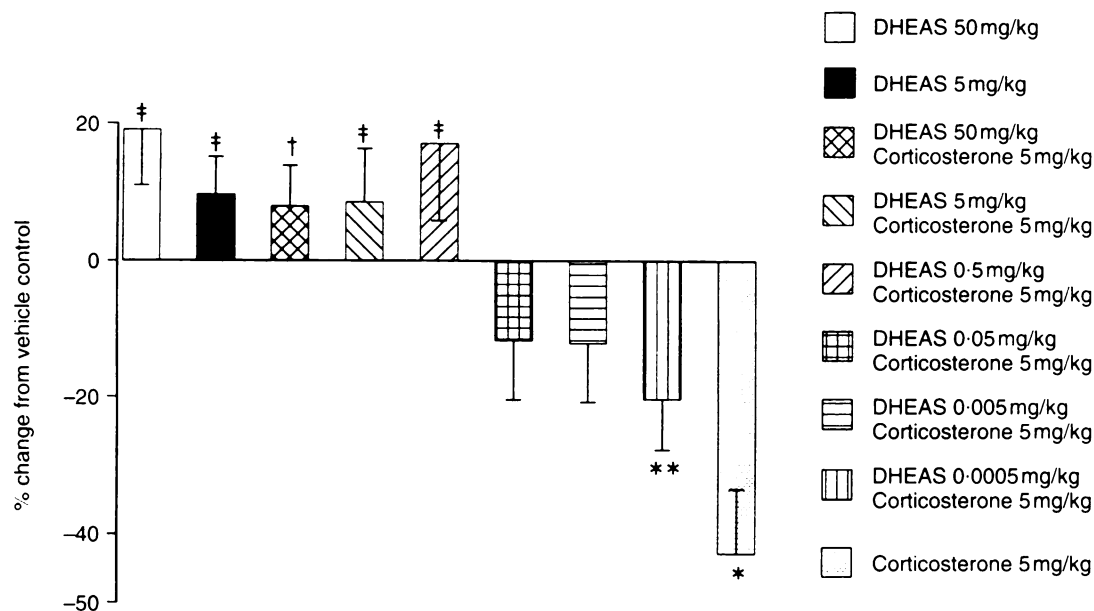


Figure 2. Reversal of corticosterone inhibition with DHEAS. Animals were sensitized with 2.5% oxazalone on day 0, and challenged with 0.25% oxazalone on day 5. Steroids were given on day 0 and 4 s.c. Ear measurements were taken at 24 h postchallenge, and expressed as percentage change from vehicle control. Change in ear thickness from prior measurement in vehicle control = 0.185 mm and challenge only control = 0.0095 mm. Mean \pm SEM. $n = 10$ per group. * $P < 0.05$ from vehicle control. ** $P < 0.05$ from DHEAS 50 mg/kg. † $P < 0.01$ and ‡ $P < 0.001$ from corticosterone.

Table 1. Dose response to CT2251. Animals were sensitized with 2.5% oxazalone on day 0, and challenged with 0.25% oxazalone on day 5. CT2251 was given on day 0 and 4 s.c. Ear measurements were taken at 24 hr postchallenge, and expressed as percentage change from vehicle control. Change in ear thickness from prior measurement in vehicle control = 0.136 mm and challenge only control = 0.0075 mm. Mean \pm SEM (in brackets). $n = 7-14$ per group

	CT2251					
	0.3 mg/kg	0.1 mg/kg	0.03 mg/kg	0.01 mg/kg	0.003 mg/kg	0.001 mg/kg
Change in ear thickness from prior measurement (mm)	0.081* (0.045)	0.083* (0.006)	0.128 (0.007)	0.131 (0.006)	0.135 (0.009)	0.128 (0.011)
% inhibition from vehicle control	42.93* (14.43)	40.94* (5.37)	5.84 (5.79)	3.89 (5.45)	0.825 (7.36)	5.75 (8.87)

* $P < 0.05$ from vehicle control.

lenged, and, treated with vehicle, the water content in the ear was increased to 69.7%. There was no effect of treatment with DHEAS \pm CT2251 or DEX on water content within the ear.

DISCUSSION

In the present study we have investigated the effect of the steroid hormones, DHEA and DHEAS, and, the role played by SS *in vivo* on a T cell-dependent model of immune function and inflammatory response. We have reproducibly demonstrated that both DHEA and DHEAS augment the CS-induced ear swelling response in mice, whereas GC, such as DEX and corticosterone, inhibit the response. These results are consistent with the observations of Daynes *et al.*,¹⁴⁻¹⁷ who presented evidence from *in vitro* and *ex vivo* data that GC down-regulated IL-2, and increased IL-4 production (i.e. up-regulating a Th2-type response). In contrast, DHEA acts by increasing the production of IL-2 from T cells, and increas-

ing proliferation of these cells (i.e. up-regulating a Th1-type response). In addition, they suggested that DHEA exerts its effects directly on the T cells, since DHEA exposure of APC was without effect¹⁴ and DHEA receptors have been found in T cells.²⁶ However more recent data suggests the presence of a DHEA receptor in monocytes.²⁷ Nevertheless Daynes *et al.*^{14,16} concluded that DHEA acts as an immunostimulant, whereas GC act as an immunosuppressant during immune responses. Moreover these and other authors suggested that immune responses may be influenced by opposing effects of these two types of steroid in a counter-regulatory fashion.^{14,16,18,28}

Although the exact mechanism of action of DHEA remains unclear, the consistency of their *in vitro* data with our results, suggests that the effect of DHEA *in vivo* may be similar, by potentiating the production of Th1-type cytokines and increasing proliferation of T cells. In CS (a T cell-dependent Th1 immune reaction) these effects would certainly lead to a

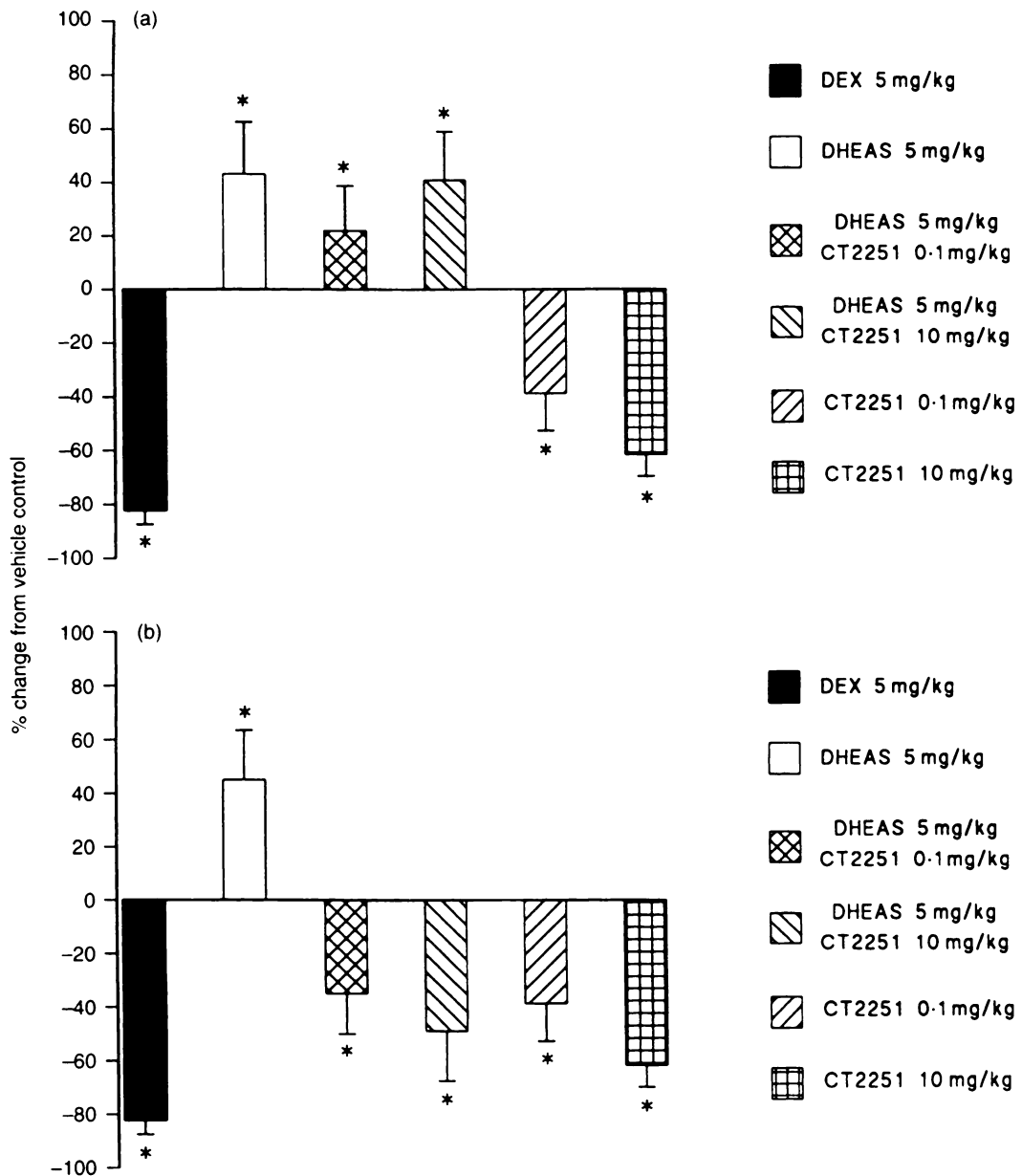


Figure 3. Effect of SS inhibitor, CT2251, on DHEA-augmented responses (a) and DHEAS-augmented responses (b). Animals were sensitized with 2.5% oxazolone on day 0, and challenged with 0.25% oxazolone on day 5. Steroids were given on day 0 and 4 s.c. Ear measurements were taken at 24 h postchallenge, and expressed as percentage change from vehicle control. Change in ear thickness from prior measurement in vehicle control = 0.104 mm and challenge only control = 0.0057 mm. Mean \pm SEM. $n = 7$ per group. * $P < 0.05$ from vehicle control.

positive effect on the ear swelling end-point of the response. Moreover, Suzuki *et al.*²⁹ have shown that in patients with systemic lupus erythematosum (SLE), defects of IL-2 synthesis from T cells correlated with low serum DHEA levels. Addition of exogenous DHEA restored the impaired IL-2 production of T cells from these patients *in vitro*. Indeed, our results suggest that immune responses *in vivo* can be influenced by the opposing effects of DHEA, DHEAS and GC. In support, we have shown that the balance between DHEA, DHEAS and GC will affect the subsequent inflammatory response, such that increasing the concentration of DHEAS will reverse corticosterone inhibition of the CS reaction. This is in agreement with the work of several groups showing that DHEA

antagonizes the suppressive effect of DEX both *in vitro*, *ex vivo* and *in vivo*, with the effect of DHEA being dominant.^{14,16,18,30-32}

Conversion of DHEAS to DHEA is necessary to ensure biological activity since DHEAS is itself inactive. Therefore the converting enzyme responsible (SS) may play an important regulatory role in immune response. Although others and ourselves have shown that biological effects can be obtained by administering exogenous DHEAS to mice, i.e. by increasing IL-2 levels^{14,16} or increasing CS response (this paper), only by inhibiting the activity of the SS enzyme itself can a regulatory role be confirmed, possibly by allowing endogenous GC effects to predominate. We have shown that an inhibitor of SS,

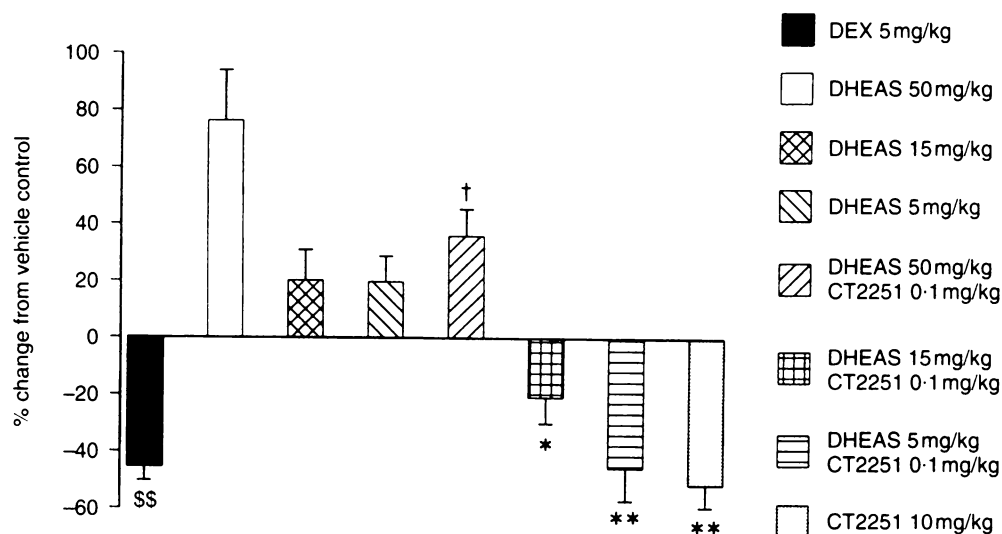


Figure 4. Reversal of CT2251 inhibition by DHEAS. Animals were sensitized with 2.5% oxazalone on day 0, and challenged with 0.25% oxazalone on day 5. CT2251 and DHEAS was given on day 0 and 4 s.c. Ear measurements were taken at 24 hr postchallenge, and expressed as percentage change from vehicle control. Change in ear thickness from prior measurement in vehicle control = 0.114 mm and challenge only control = 0.0057 mm. Mean \pm SEM. $n = 7-14$ per group. * $P < 0.05$ from DHEAS 50 mg/kg, ** $P < 0.01$ from DHEAS 50 mg/kg, † $P < 0.05$ from DHEAS 5 mg/kg + CT2251 0.1 mg/kg and CT2251 0.1 mg/kg, ‡ $P < 0.01$ from DHEAS 50 mg/kg and DHEAS 50 mg/kg - CT2251 0.1 mg/kg.

Table 2. Effect of (a) DHEAS, DEX and (b) CT2251 on cellular infiltration in the ear. Animals were sensitized with 2.5% oxazalone on day 0, and challenged with 0.25% oxazalone on day 5. DHEAS (5 mg/kg), DEX (5 mg/kg) and CT2251 (0.1 mg/kg) were given on days 0 and 4 s.c. Ear measurements were taken at 24 hr postchallenge. (a) Change in ear thickness from prior measurement in vehicle control = 0.107 mm and challenge only control = 0.0065 mm, and (b) vehicle control = 0.221 mm and challenge only control = 0.008 mm. Ears were removed for staining for the presence of T cells (CD3⁺) and macrophages (Mac-1⁺)

	T cells per mm skin	Macrophages per mm skin
(a)		
Challenge only	0.51 (0.169)	0
Vehicle control	23.81 (3.344)	93.82 (13.39)
DHEAS	39.00* (2.455)	194.23* (26.98)
DEX	7.91** (2.681)	9.31** (2.88)
Normal	1.44 (0.329)	0.70 (0.16)
(b)		
Vehicle control	36.2 3.83	131.67 16.39
CT2251	12.56† (2.02)	25.99† (7.54)

Mean \pm SEM (in brackets). (a) $n = 9-10$ per group; * $P < 0.001$ from all groups, ** $P < 0.001$ from all groups except challenge only and normal. (b) $n = 19-20$ per group, † $P < 0.001$ from vehicle control.

CT2251, has activity *in vivo*, demonstrating inhibition of liver steroid sulphatase activity for a number of days following administration. In addition, CT2251 will inhibit a CS response *in vivo*, when given on its own. Therefore without the addition

of exogenous DHEA/DHEAS, inhibition of SS will attenuate an ongoing immune response, presumably by preventing conversion of endogenous DHEAS to DHEA. Furthermore, SS inhibitor reversed a DHEAS-augmented response, but not a DHEA-augmented response. Since only DHEAS is the substrate for the enzyme, this would suggest that SS plays an important physiological role in regulating immune responses.

It has been shown that much of the effect of DHEA in augmenting a Th1-type response is mediated in the secondary lymphoid organs, with particularly marked effects in those lymphoid organs which normally give rise to high IL-2 and interferon- γ (IFN- γ) cytokine profiles, such as spleen and lymph nodes.¹⁶ The effect of DHEA is less marked in Peyer's patches, which show a predisposition to produce IL-4. Lymphoid organs showed a direct correlation between high expression of SS activity and ability to produce IL-2. More recent evidence by Hennebold and Daynes³³ demonstrated that most SS activity resided within the macrophage population. Therefore it is reasonable to speculate that conversion of DHEAS to DHEA occurs through the activity of SS within macrophages, which itself has a direct effect on T cells, leading to increased production of IL-2 within the local environment of the spleen or relevant lymph nodes, thus potentiating the immune response.

The CS [delayed-type hypersensitivity (DTH)-like] response results, upon sensitization of the animals, in an inflammatory end-point, namely ear swelling.³⁴ Because of the profound effects of DHEAS and CT2251, we were interested in their effects on the mechanism of ear swelling. Ear challenge of sensitized animals did not cause much oedema, suggesting that fluid influx is not an important component of this response, and none of the treatments used had any effect. This would agree with the evidence demonstrating that a DTH immune response consists primarily of a cellular infiltrate causing a hard induration.³⁴ In contrast to the effect on oedema, treatment with DHEAS and CT2251 had a marked effect on the cellular infiltrate. Correlating with the increase

Table 3. Effect of steroids and CT2251 on oedema in the ear. Animals were sensitized with 2.5% oxazolone on day 0, and challenged with 0.25% oxazolone on day 5. Steroids, DHEAS (5 mg kg) and DEX (5 mg kg), and CT2251 (10 mg kg) were given on day 0 and 4 s.c. Ear measurements were taken at 24 hr postchallenge, and the ears removed for assessment of water content. Change in ear thickness from prior measurement in vehicle control = 0.118 mm and challenge only control = 0.0053 mm. Mean \pm SEM (in brackets), $n = 10$ per group

	Normal	Challenge only	Vehicle control	DEX	DHEAS	DHEAS - CT2251	CT2251
Ear water content	55.4* (0.667)	61.47* (0.4)	69.70 (0.799)	68.89 (0.69)	70.37 (0.522)	71.15 (0.758)	70.88 (0.761)

* $P < 0.001$ from all groups.

in ear swelling response, DHEAS increased the number of both immune (T cells) and inflammatory (monocytes macrophage) cells. This increase could occur as a direct result of the potentiated production of IL-2. This is a cytokine produced by activated T cells, which plays a critical role in T-cell biology, stimulating the further proliferation of activated cells, and the secretion of other cytokines such as IFN- γ , IL-1 and tumour necrosis.³⁵ In particular IFN- γ is a known activator of macrophage function and other cells, leading to an influx of these cells to the site of an immune reaction. In contrast, and again correlating with the ear swelling results, CT2251 reduced cellular infiltration. By inhibiting the conversion of DHEAS to DHEA, CT2251 may be having an indirect effect on the cytokine cascade, thus affecting the stimulus for cellular activation, proliferation and migration.

In conclusion, we have shown that DHEA is both a pro-immune and pro-inflammatory steroid hormone with the capacity to potentiate an immune response *in vivo*. The conversion of DHEAS to DHEA by the SS enzyme is necessary to allow this effect to occur. Although the exact mechanism of action is unproven *in vivo*, the potential for a therapeutic use of SS inhibitors is clear.

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